

First Quarterly Report for:
An Exploration of the Use of Parasitic Nematodes for the Biological Control of Variable Leafed Milfoil
(*Myriophyllum heterophyllum*)

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Project Activities to Date:

Contract edits were finalized and upon notification of Governor and Council approval. The UNH project start date was May 4, 2005 a few months after the originally expected start time. Also in May the principal investigators met to plan out our sampling strategy, reset our project calendar, and compile methods and procedures for QAPP development.

From May through June, working with Wisconsin DNR, Wisconsin Biological Survey and using datasets from the Wisconsin LTER lakes program and the computerized records from the University of Wisconsin Herbarium a list of about 20 candidate lakes containing our target species were selected. When possible, DNR staff provided site location information. Note: In April, contact was also made with Minnesota DNR staff who also were to provide candidate lake information. Even though our request clearly stated our target species was *M. heterophyllum* they sent us a list of lakes containing *M. spicatum*. Unfortunately they reported back in June that *M. heterophyllum* is not considered an exotic and thus is not documented by their staff.

In June we planned our trip itineraries to Wisconsin based on local information on when to expect flowering of the milfoil (late July through August). We secured permits from NH Fish and Game for importing and culturing nematodes after preparing a containment protocol. We secured an import permit for *Myriophyllum* species from NH DES. Nematode plant extraction apparatus (mist chambers) were constructed at the Hubbard Center. All PIs and two technicians took part in cross-training to prepare for sampling.

In July we held two shake down sampling trips at Squam (Squam River and Bennet Cove) and Wentworth Lakes (Heath Tributary inlet area). This gave us different sediment conditions to try plant extraction, sediment sampling and soil water collection techniques. It also allowed for training the lab technician to do the nematode extractions in preparation of receiving the overnight shipped samples from the Midwest.

In August a preliminary survey trip to the candidate Wisconsin lakes was made by Schloss and Crow (whose travel expenses were mostly covered under another grant). Of thirteen lakes surveyed, *M. heterophyllum* was only confirmed at two lakes: Rock Dam and Round Lake. There were occurrences of other milfoil species which may have been mistakenly identified, or the site locations (if provided) were not accurate and we failed to find occurrences, or the populations reported may no longer occur. A second trip to Wisconsin was made by Schloss and Abebe to collect additional samples at Rock Dam and Round Lakes and to survey two additional lakes suggested by Wisconsin DNR staff. Due to poor weather conditions (lightning storms) we were only able to sample the confirmed lakes and one of the additional lakes. We since have set up a DNR contact who will provide us with samples in Spring of 2006 from the missed lake.

Nematode extractions were done through the summer with morphological type separations and sorting occurring in the fall and winter. Preliminary results are reported in a later section.

QAPP development continued through the summer and fall as techniques and protocols were tested for inclusion. A draft QAPP was provided to NH DES in October. Comments from NH DES were returned at the end of November. Edits were completed and a 2nd revision QAPP was sent to EPA New England for approval in January.

Summary of Plant and Community Analysis (preliminary):

More detailed field notes from the first Wisconsin surveys have already been transmitted to NH DES directly. While most of the NH analysis will be done in 2006 the following are just some preliminary observations to use as context for the nematode results. Wentworth and Squam had very different community structure. Squam populations sampled tended to be clustered as if each cluster was from a single original plant that spread out concentrically. This was especially true of the Squam River sites. Squam populations were extremely monospecific. Wentworth populations were more evenly distributed and had occasional occurrences of other tall submergent plants including two species of Potamogeton. The Wentworth milfoil also seemed to occupy a more specific range of depth just beyond the floating and emergent plant “fringe” and up to about 6 to 7 feet depth. The Wisconsin plants occurred in more shallow waters and were often found among floating leaved species. They occurred as a very small cluster or small patches along with other submerged and emergent plants.

Summary of Work Done on Nematodes:

We have collected multiple sediment core samples and plant material from four lakes: two lakes in New Hampshire (Wentworth and Squam) and two lakes in Wisconsin (Rock Dam and Round). Sediment core samples were kept in an iced cooler and transported to Durham for extraction. Each sample was divided into plant material and rhizosphere sediment. We followed three methods for nematode extraction: mist chamber and Whitehead trays for the extraction of nematodes from plant material, and the centrifugal floatation method for the extraction of nematodes from sediments.

Extraction produced nematodes in all sediment collected; the least number of nematodes were found from Squam. We picked and transferred all nematode specimens into 20% dimethyl sulfoxide (DMSO), a recently developed method for the preservation of primarily marine nematodes. We have tested the method's efficiency on our samples and it has yielded successful results. We have picked 100 nematodes from the Wentworth Heath Tributary inlet and have subsequently identified 14 genera. Samples were not preserved in DMSO in the case of the identified nematodes from Wentworth because the method was developed later. The diversity we found in Squam lake was low; only 3 genera. We also have picked 100 nematodes from each of Rock Dam Lake and Round Lake and have prepared slides mounted in DMSO in preparation for imaging and detail morphological study. Following morphological identification, we will extract DNA from these specimens and will sequence target genes from all samples this winter.

Upcoming Work:

We look forward to reviewing the preliminary results of the other projects done this past summer. Nematode genetics work ups and cataloguing will continue through the winter and we will start preparing for sampling NH and WI lakes in the spring. We expect to sample through the summer of 2006.